W.S. Research Report
66
#66

# EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

LIBRARY
MAY 13 1965
FISH & WILDLIFE SERVICE

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 66

## RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two Bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

Current Research Reports are—

54. Fluctuations in Age Composition and Growth Rate of Cutthroat Trout in Yellowstone Lake, by Ross V. Bulkley. 1961. 31 p.
55. Mortality Studies on Cutthroat Trout in Yellowstone Lake, by Orville P. Ball and Oliver B. Cope. 1961. 62 p.
56. Limnology of Yellowstone Lake in Relation to the Cutthroat Trout, by Norman G. Benson. 1961. 33 p.
57. Chemical Composition of Blood of Smallmouth Bass, by Eddie Wayne Shell. 1961. 36 p.
58. Chronic Effects of Endrin on Bluntnose Minnows and Guppies, by Donald Irvin Mount. 1962. 38 p.
59. Predicting Year-Class Abundance of Yellowstone Lake Cutthroat Trout, by Ross V. Bulkley and Norman G. Benson. 1962. 21 p.
60. Limnological Organic Analyses by Quantitative Dichromate Oxidation, by John A. Maciolek. 1962. 61 p.
61. Bioassay and Use of Pituitary Materials to Spawn Warm-Water Fishes, by Howard P. Clemens and Kermit E. Sneed. 1962. 30 p.
62. Equilibrium Yield and Management of Cutthroat Trout in Yellowstone Lake, by Norman G. Benson and Ross V. Bulkley. 1963. 44 p.
63. Sea-Water Systems for Experimental Aquariums; A Collection of Papers, edited by John R. Clark and Roberta L. Clark. 1964. 190 p.
64. Some Chronic Effects of DDT on Cutthroat Trout, by Don Allison, Burton J. Kallman, Oliver B. Cope, and Charles Van Valin. 1964. 30 p.
65. Revised Bibliograph on the Cutthroat Trout, by Oliver B. Cope. 1964. 43 p.
66. Effects of Accumulated Excretory Products on Hatchery-Reared Salmonids, by Roger E. Burrows. 1964. 12 p.
67. A Device for Stamina Measurement of Fingerling Salmonids, by Allan E. Thomas, Roger E. Burrows, and Harry H. Chenoweth. 1964. In press.

# EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

Roger E. Burrows

*Fishery Research Biologist*

RESEARCH REPORT 66

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, *Secretary*

Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

FISH AND WILDLIFE SERVICE

Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE

Daniel H. Janzen, *Director*

Published by the Bureau of Sport Fisheries and Wildlife • Washington • 1964

Printed at the U.S. Government Printing Office, Washington, D.C.

## CONTENTS


| | Page |
|---|---|
| Introduction | 1 |
| Principal excretory products in rearing ponds | 1 |
| Effects of excretory products on the fish | 4 |
| Conclusions | 10 |
| Literature cited | 11 |

## ABSTRACT

In experiments at the Bureau's Salmon-Cultural Laboratory, to determine the excretory products of significance in rearing ponds and the possible effect of these products on fingerling chinook salmon (*Oncorhynchus tshawytscha*), the results demonstrate that ammonia and urea are the principal products. The relative amounts of these products vary with the concentration of fish and with their metabolic activity. Ammonia assumes dominance in an unfavorable environment. The pH, water temperature, and pond type affect the toxicity of ammonia solutions. Continuous exposure to levels of ammonia normally encountered in rearing ponds produced extensive hyperplasia of the gill epithelium in controlled experiments. In rearing ponds, prolonged but not continuous exposure in unionized ammonia has been demonstrated to result in reduction both in growth rates and in physical stamina. It is postulated that the gill hyperplasia resulting from continuous exposure to un-ionized ammonia is a precursor of bacterial gill disease.

# EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

**By Roger E. Burrows, *Fishery Research Biologist***

Salmon-Cultural Laboratory, Bureau of Sport Fisheries and Wildlife
Longview, Washington

This is the second report on factors that limit production in rearing ponds. It is concerned principally with excretory products, their accumulation, and their effects on hatchery-reared salmonids.

The first report, by Burrows and Chenoweth (1955), was on investigations designed to explore the hydraulic and biological characteristics of several types of rearing ponds. Its principal purpose was to define the problem. The results emphasized the lack of information applicable to the measurement of environmental effects and the need for more intensive investigations into the limitation imposed by water quality, excretory products, water temperature, pond types, and the reuse of water.

The effect of excretory products was selected as the second study because it is not only a relatively unexplored field but also one of the most critical hatchery problems. The method of attack was first to define the significant excretory products in rearing ponds, and second to measure the effect of these products on the fish.

## PRINCIPAL EXCRETORY PRODUCTS IN REARING PONDS

Salmon and trout rearing ponds vary in size and shape, but all are supplied continuously with water. The problem of measurement of the excretory products is complicated by this constant interchange. Most investigators have confined their experiments to aquariums which allow the excretory products to accumulate, thus leading to erroneous conclusions about the normal relations of certain products of excretion.

Ammonia nitrogen has been found to be one of the principal products of excretion. Because of the confusion of terminology in the literature, this term needs further definition. According to dictionary definition (Webster, 1951) ammonia itself is the gas $NH_3$, and ammonia nitrogen is nitrogen combined in the form of ammonia, $NH_3$, or ammonium, $NH_4$. The ammonium radical $NH_4$ combines with a number of elements, but as a product of excretion it is present as ammonium hydroxide which dissociates in a reversible reaction into ammonia, $NH_3$, and water. In some of the more recent literature (Wuhrmann and Woker, 1949; Downing and Merkins, 1955; Merkins and Downing, 1957; and Lloyd and Herbert, 1960) ammonia and ammonia nitrogen are used

almost as synonyms, with the term ammonia referring to aqueous ammonia containing both the gas, $NH_3$, and ammonium hydroxide, $NH_4OH$. The ammonia gas, $NH_3$, is identified, specifically, as un-ionized ammonia. This terminology will be used in this report. Most of the earlier work considers ammonia in the same sense with the exception that the significance of the un-ionized form is not recognized.

Smith (1929) found that the principal end products of catabolism in carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were ammonia and urea. Wood (1958) found that in three species of marine fishes 75 to 98 percent of the total nitrogen excreted consisted of ammonia and urea. Both Smith and Wood analyzed the water from aquariums in which fish had been held for 18 to 24 hours to determine the products of excretion. Both workers found that the amount of ammonia excreted was much greater than that of urea.

Our approach was different in that hourly samples of water from rearing pond effluents were analyzed over 24-hour periods to determine the significant products of excretion. Our problem, of course, was different in that we were interested primarily in how the products of excretion affected the fish in rearing ponds.

Analyses of the effluents were made for ammonia, urea, creatinine, and uric acid; ammonia by direct nesslerization (Ellis et al., 1946), urea by the Karr method (Karr, 1924), creatinine by the method of Folin and Wu (1919), and uric acid by the method of Folin (1933, 1934). Urea and ammonia were the major excretory products as these were the only products which appeared in measurable amounts. Contrary to both Smith's and Wood's results, either ammonia or urea could be the principal excretory product dependent either on the size and weight of fish in the pond or on the time of day of the analysis.

The effluent concentrations of ammonia and urea from several pond types were measured in a series of experiments. The results of one of these evaluations is shown in figures 1 and 2. In this experiment, two 4- by 40-foot raceway ponds were stocked with equal poundages of comparable chinook salmon (*Oncorhynchus tshawytscha*) fingerlings. The water inflow into one pond was half that of the other, 125 gallons per minute as compared with 250 gallons per minute. A similar difference in the ratio of fish weight to water inflow was maintained throughout a growing season as the total weight of fish increased. At intervals of approximately 6 weeks, the effluent waters were analyzed for both ammonia and urea at hourly intervals for 24 hours. The results of these analyses for the high inflow pond are shown in figure 1. With the low loading rate used in this pond, urea was the principal product of excretion throughout the season. At the higher loading rate, and an average water temperature of 52° F. (fig. 2), ammonia soon became the predominant excretory product. This dominance was demonstrated at a loading rate of 5.6 pounds of fish per gallon per minute of inflow. At higher metabolic rates, influenced both by fish size and by water temperature, ammonia dominance would be expected at a lower loading rate. At the end of August as shown in figure 1, ammonia dominance was approached in the raceway with the higher inflow, when the temperature reached 55° F. and the weight of fish per gallon per minute of inflow was 4.37 pounds.

The physiological processes involved in the suppression of urea excretion have not been explored. Certainly no reference to this phenomenon has occurred in the literature principally because the experi-

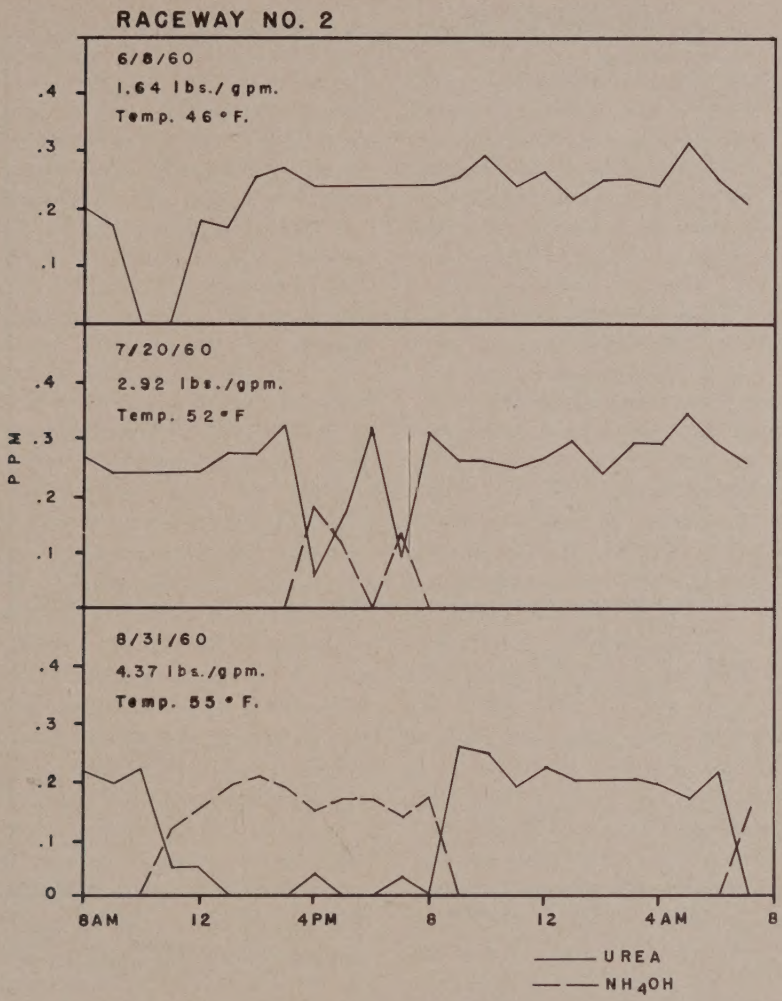


FIGURE 1.—Patterns of ammonia and urea excretion in a raceway pond with high inflow. Ammonia nitrogen expressed as $NH_4$.

mental procedures employed accumulated the excretory products in small quantities of water for comparatively long periods of from 18 to 24 hours with aeration but without water replenishment. The results of such conditions are similar to those encountered at the 8.16 pound loading rate shown in figure 2 in which ammonia predominated and urea was present in very small quantities.

Although figure 1 shows no ammonia excretion at the low loading rate of 1.64 pounds, such was probably not the case. The direct nesslerization method for the measurement of ammonia nitrogen is not accurate below 0.1 p.p.m. Thus, small quantities of ammonia could have been and probably were being excreted by the fish even though no ammonia was indicated by the measurements.

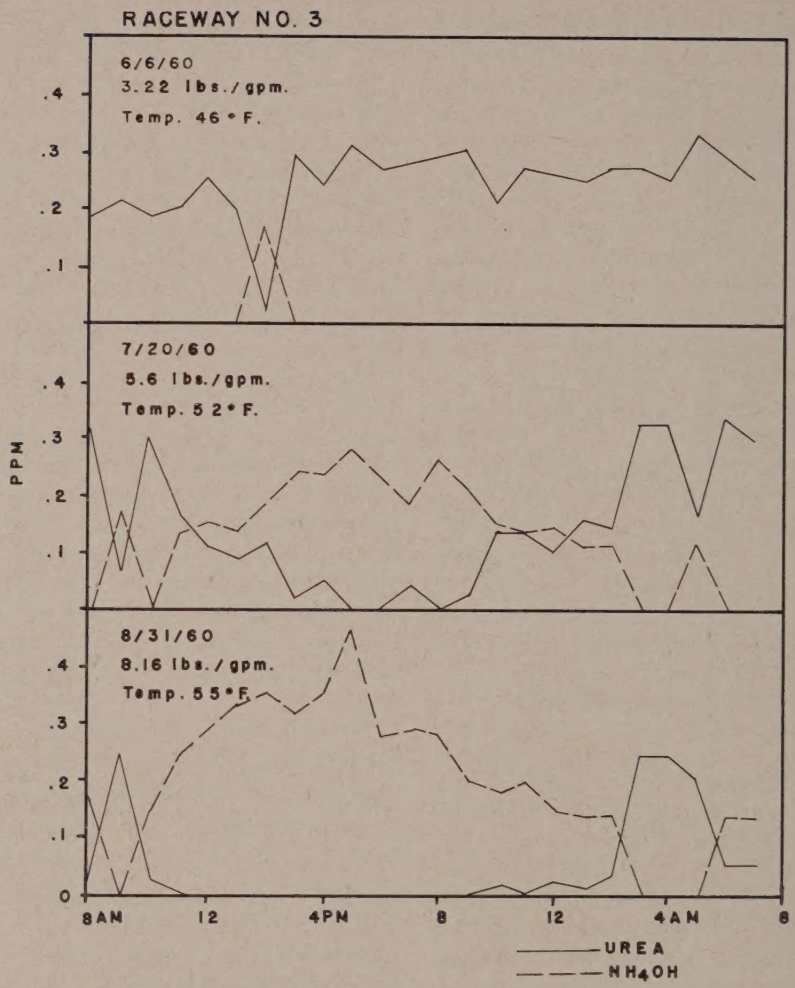


FIGURE 2.—Patterns of ammonia and urea excretion in a raceway pond with low inflow. Ammonia nitrogen expressed as $NH_4$.

## EFFECTS OF EXCRETORY PRODUCTS ON THE FISH

Although various pond types studied over a 10-year period differed in their expulsion rates and therefore in the rate of removal of waste products from the pond water, the domination of ammonia was characteristic of high loading rates in all tests. Since fish mortality is also associated with very high loadings, ammonia was suspect as a toxin. In several experiments where overloads were associated with outbreaks of gill disease, ammonia was present continuously in the pond effluent and urea was either unmeasurable or present in but small quan-

tities and for but short periods of time.

To measure the effect of ammonia under controlled conditions, two experiments were conducted for a period of 6 weeks, at water temperatures of 43° F. and 57° F. In each experiment, four lots of chinook salmon fingerlings were subjected to constant levels of introduced ammonium hydroxide at 0.0, 0.3, 0.5, and 0.7 p.p.m., calculated as $NH_4$. The highest experimental level approximates the maximum concentration encountered in rearing ponds during afternoon surges.

Effects of ammonia on gills of fish held at 43° F. are shown in figures 3 to 6. Figure 3 is a photomicrograph of a typical gill section from the control group showing no proliferation of the gill epithelium. In figures 4 through 6 the correlation of proliferation with exposure to higher ammonia concentrations is obvious. These sections were selected as generally typical of the samples examined. At both temperatures some fish in all the samples from the ammonia-treated groups showed the extensive proliferation and clubbing of the filaments characteristic of the highest ammonia concentration. Sample checks at weekly intervals throughout both of the experiments indicated that the degree of proliferation was progressive for the first 4 weeks, after which no measurable increases were discernible.

There was no significant mortality during the experiments and for an additional 3 weeks afterwards. Examination of a sample of the low-temperature fish after 3 weeks in fresh water indicated that no recovery from the extensive proliferation had taken place. In the second experiment, conducted at 57° F. with larger fish, a marked recovery from the hyperplasia was noted after 3 weeks' exposure to fresh water. In the first experiment, the proliferated areas had consolidated; in the second, they had not.

In a third experiment, urea at 0.2, 0.3, and 0.4 p.p.m. was substituted for ammonia. Continuous exposure at these concentrations for a 3-week period resulted in no indication of proliferation of the gill epithelium in the fish, and the experiment was terminated. As the highest urea concentration exceeded the maximum level encountered in rearing ponds and the exposure period was beyond that when ammonia produced visible evidence of gill irritation, it was concluded that ammonia, not urea, was the toxicant.

The toxicity of ammonia solutions is determined by the amount of un-ionized ammonia present (Wuhrmann and Woker, 1949). The degree of dissociation is controlled primarily by the pH and the temperature of the solution. The toxicity tests on ammonia were conducted at water temperatures of 43° F. and 57° F. and at a pH of 7.8. In these tests the calculated exposure to un-ionized ammonia was 0.006 and 0.008 p.p.m. at the 0.3 p.p.m. ammonium hydroxide level; 0.010 and 0.012 p.p.m. at the 0.5 p.p.m. level; and 0.014 and 0.018 p.p.m. at the 0.7 p.p.m. level.

Most investigations have been concerned with the lethal effects of ammonia concentrations on the fish. Lloyd and Herbert (1960) have demonstrated that the free carbon dioxide in the water can cause a decrease in the pH value and the amount of un-ionized ammonia at the gills. Downing and Merkins (1955) and Merkins and Downing (1957) demonstrated that the lethality of un-ionized ammonia increased with a reduction in dissolved oxygen. This is consistent with the work of Phillips et al. (1949) and Brockway (1950) in which a reduction in the oxygen level of the blood was correlated with an increase in the catabolic products in the water.

Lloyd and Herbert report the lethal level of un-ionized ammonia at a pH of 8.2

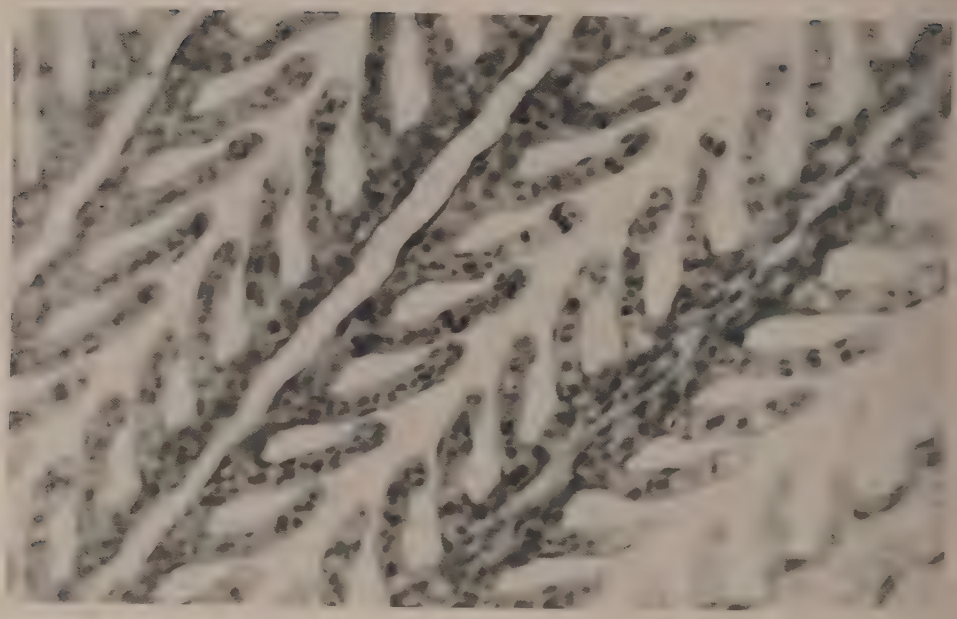

FIGURE 3.—Normal gill section from control group.

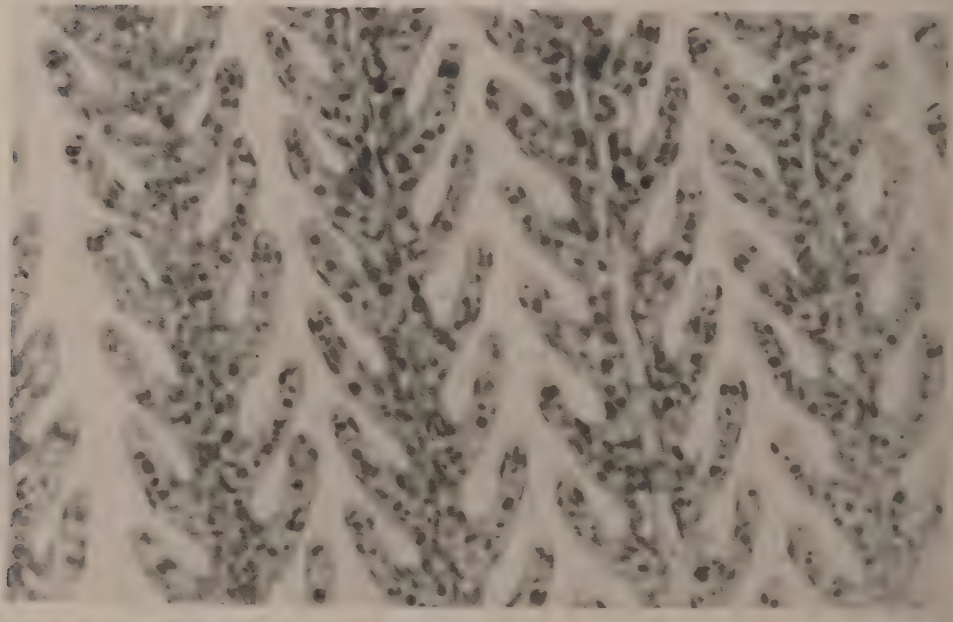

FIGURE 4.—Typical gill section from group exposed to 0.3 p.p.m. of ammonium hydroxide for 6 weeks showing proliferation of the gill epithelium.

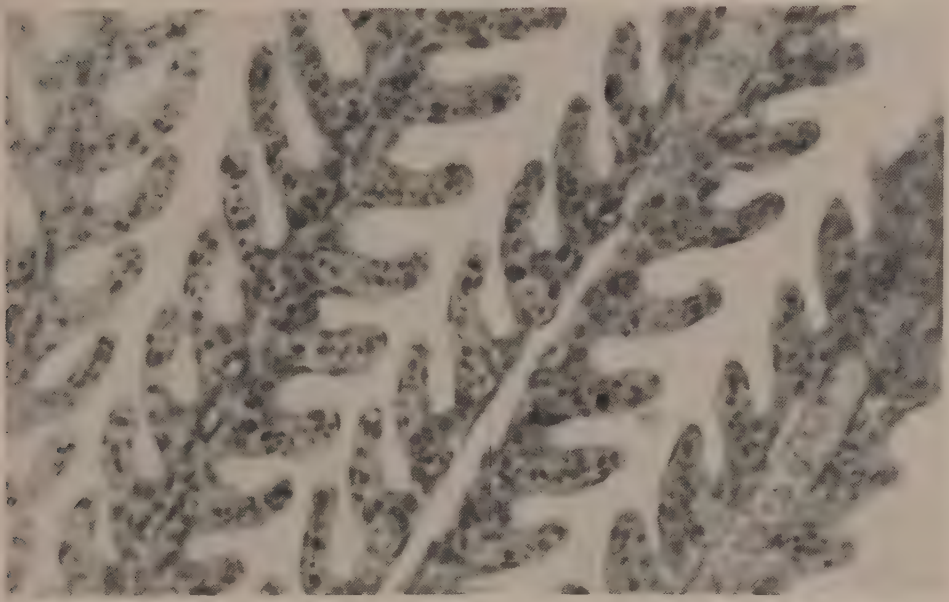

FIGURE 5.—Typical gill section from group exposed to 0.5 p.p.m. of ammonium hydroxide for 6 weeks showing extensive hyperplasia.

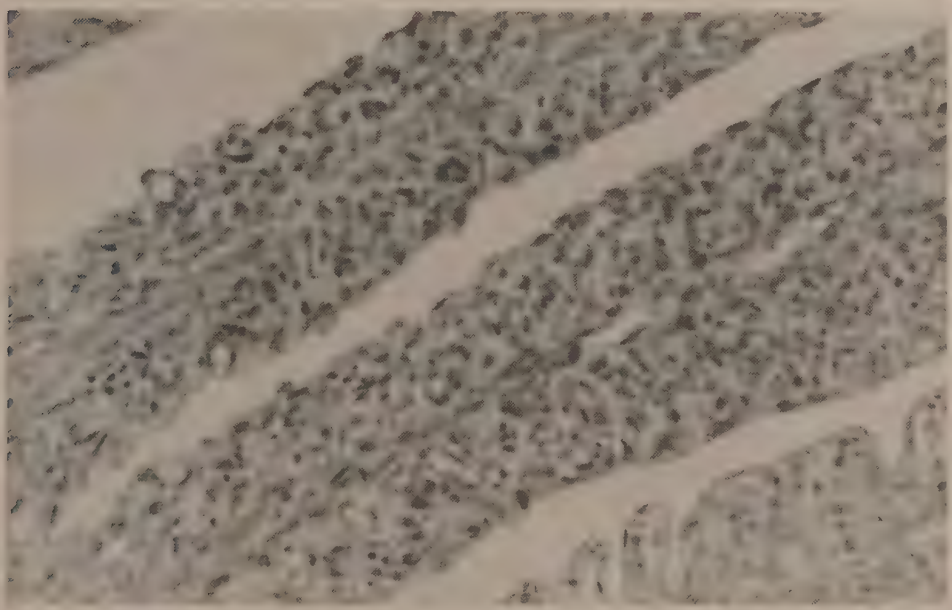

FIGURE 6.—Typical gill section from group exposed to 0.7 p.p.m. of ammonium hydroxide for 6 weeks showing fusion of lamellae. (Photomicrographs by W. T. Yasutake.)

and normal levels of oxygen and carbon dioxide to be 0.84 p.p.m. At a water temperature of 50° F., 18 p.p.m. of ammonia nitrogen expressed as $NH_4$ would be necessary to produce a lethal effect in rainbow trout (*Salmo gairdneri*). Such a concentration is highly unlikely in salmon and trout rearing ponds unless the water is being extensively reused without reconditioning.

The toxic levels of ammonia, other than actual lethal levels, are difficult to ascertain primarily because the effects of ammonia exposure are insidious and indirect. The literature is confusing because most authors merely give the concentration of ammonia found in the pond water, when describing toxic or lethal effects. As the toxin is the un-ionized ammonia present, the toxic concentrations cannot be calculated unless the water temperature and pH are known. Table 1 lists the percentages of un-ionized ammonia contained in ammonium hydroxide solutions at three temperatures and the pH levels normally encountered in salmonoid rearing. From table 1 it will be noted that insignificant levels of the gas are present at a pH of 7 but that the proportion of un-ionized, i.e. toxic, ammonia progressively and markedly increases with the increase of the pH of the aqueous solution, whereas the influence of temperature on the rate of dissociation is comparatively minor. The correlation between pH and un-ionized ammonia explains the vast differences encountered in the carrying capacities of different water supplies.

The effects of even low concentrations of un-ionized ammonia are real, demonstrable, and definitely detrimental. From accumulated evidence it can be demonstrated that, when un-ionized ammonia concentrations become dominant in rearing ponds, growth rate, physical stamina, and disease resistance are impaired.

TABLE 1.—*Percentage of un-ionized ammonia in ammonium hydroxide solutions at three temperatures and various levels of pH*

[Prepared by J. W. Elliott after Wuhrmann and Woker (1949)]

| pH | At 10° C. (50° F.) | At 15° C. (59° F.) | At 20° C. (68° F.) |
|---|---|---|---|
| 7.0 | 0.3 | 0.4 | 0.5 |
| 7.5 | 1.1 | 1.3 | 1.5 |
| 7.6 | 1.4 | 1.6 | 1.9 |
| 7.7 | 1.8 | 2.1 | 2.4 |
| 7.8 | 2.3 | 2.6 | 3.0 |
| 7.9 | 2.9 | 3.3 | 3.8 |
| 8.0 | 3.6 | 4.1 | 4.7 |
| 8.1 | 4.6 | 5.2 | 6.0 |
| 8.2 | 5.7 | 6.5 | 7.3 |
| 8.3 | 7.1 | 8.0 | 9.1 |
| 8.4 | 8.9 | 9.9 | 11.2 |
| 8.5 | 11.1 | 12.3 | 13.7 |
| 8.8 | 20.3 | 22.1 | 24.2 |
| 9.0 | 29.1 | 32.3 | 35.8 |
| 9.5 | 57.6 | 59.8 | 62.1 |

Derivation formula: $\frac{(NH_4^-)(OH^-)}{(NH_3 \cdot H_2O)} = K_b$.

$K_b$=dissociation constant aqueous ammonia.
$K_w$=ionization constant $H_2O$.

| Temperature | $K_b$ | $K_w$ |
|---|---|---|
| 10° C. | $1.570 \times 10^{-5}$ | 14.5346 |
| 15° C. | $1.652 \times 10^{-5}$ | 14.3463 |
| 20° C. | $1.710 \times 10^{-5}$ | 14.1669 |

The reduction in growth rate due to an increase in ammonia concentration is reported from warmwater fish culture (Kawamoto et al., 1957; Kawamoto, 1958, 1961) and from salmonoid culture (Brockway, 1950). These reports are concerned with the increase in ammonia occasioned by the reuse of water and demonstrate the reduction in growth rate in ponds at the lower end of a series. In the experiment from which the excretory cycles were determined, figures 1 and 2, the growth rate was also measured. The experiment was conducted for a 5-month period with ammonia becoming dominant in the low-inflow raceway during the last 3 months. The growth rate in this raceway was 9.1 percent lower than that measured in the high-inflow raceway for the 5-month period. This significant difference occurred with but a single use of the water and without either an increase in mortality, disease outbreak, or continuous exposure to measurable levels of ammonia.

An inhibition in growth rate indicates an impaired physiology. Phillips et al. (1949) and Brockway (1950) correlate the increase in ammonia concentration with a reduction in the oxygen level in the blood. Our toxicity experiments demonstrate the extensive proliferation of the gill lamellae which can be caused by exposure to ammonia. Such proliferation and consolidation reduces the surface area of the gills and therefore the ability of the animal to absorb oxygen. These factors would be expected to result in a reduction in the physical performance of the fish under conditions of stress. Stamina trials in which groups of 100 fish were subjected to fixed water velocities for measured periods of time in a stamina tunnel (Thomas et al., 1964) demonstrated a reduced performance in fish subjected to a continuous ammonia exposure in rearing ponds as contrasted with comparable fish where the ammonia exposure was intermittent. The performance index in the ammonia-exposed fish was 16 and in the control 23. A difference of 3 performance index points was significant.

Impaired physiology implies reduced disease resistance. The susceptibility of salmon fingerlings to bacterial gill disease is closely associated with the extensive epithelial hyperplasia of the gills caused by ammonia irritation. When fish are continuously exposed to measurable amounts of ammonia, bacterial gill disease epidemics are prevalent. It is postulated that ammonia irritation is a precursor of bacterial gill disease.

The accumulated evidence indicates that continuous exposure to un-ionized ammonia is definitely detrimental and should be avoided in rearing ponds. The debilitation caused by ammonia exposure is certainly not conducive to optimum survival rates after liberation.

The principal factors affecting toxic ammonia levels in rearing pond waters are pH, temperature, and the ammonia exposure period. The fact that un-ionized ammonia is the toxin precludes the possibility of generalizing on the dangerous ammonium hydroxide levels to be encountered in rearing ponds. Within rather narrow pH ranges, however, the changes in un-ionized ammonia are relatively insignificant. Our work was conducted at a pH of $7.8 \pm 0.1$, that of Phillips et al. (1950) at an initial pH of 7.8 and 7.9. This work and the summarization of the Cortland Laboratory experiments by Brockway (1950) are in essential agreement with our findings concerning the toxicity of ammonium hydroxide concentrations in rearing ponds. Our experiments indicate that the period of exposure to measurable amounts of ammonium hydroxide within a pH range of 7.8 to 8.0 is of more importance than the peak exposure concentration. Salmon fingerlings can tolerate levels of ammonium hydroxide as great as 0.7 p.p.m. for 1 hour per day with apparent impunity, but when the exposure period exceeds 12 hours per day at levels of 0.1 p.p.m. or above, the growth rate is reduced, and when continuous exposure occurs a reduction in stamina and disease resistance develops. This latter condition occurs in raceways when the fish are stocked at the rate of 8.5 pounds per gallon per minute of inflow at a water temperature of 60° F. and a pH of 7.8.

As the water temperature increases the percent of un-ionized ammonia increases and, according to Brockway (1950), the excretion rate also increases. At temperatures of 65° F., therefore, the carrying capacity of the water would be reduced to 8.0 pounds per gallon per minute of inflow or less. At pH levels above 8, the toxicity

to the fish would be greatly increased and, conversely, at lower levels it would be reduced because of the variations in the amount of un-ionized ammonia present despite the production of comparable amounts of ammonium hydroxide under both conditions.

Because the length of the exposure period affects the toxicity of un-ionized ammonia, the hydraulic characteristics and explusion rates of the various types of rearing ponds assume importance. Pond types, which develop excessive detention times either due to poor hydraulic patterns or inadequate inflows, create conditions whereby the ammonia exposure is increased beyond the normal. Burrows and Chenoweth (1955) describe three pond types and the hydraulic patterns which can create such conditions and have correlated the hydraulic characteristics with the disease inhibition qualities. Brockway (1950) emphasizes the effects of laminar flow in raceways with inadequate inflow and was able to demonstrate increased ammonia concentrations in the deeper stratified areas.

Recognition of the period when dangerous toxic levels of ammonia are approaching is essential if they are to be avoided. Examination of the excretory patterns indicates that ammonia as it approaches dominance is present in the p.m. hours and urea in the a.m. hours. As the period of ammonia excretion increases in the 24-hour cycle, the low ebb occurs in the early morning. The occurrence of measurable amounts of ammonia in the pond effluent during the period of minimum physical and metabolic activity is an indication that the fish are being subjected to a continuous ammonia exposure and an unfavorable environment.

These investigations have demonstrated that continuous exposure to un-ionized ammonia can produce a general debility. Such debility may be an important factor influencing survival of salmon and trout both before and after liberation.

## CONCLUSIONS

Experiments conducted to determine the excretory products of significance in salmonid rearing ponds and the effect of these products on the fish lead to the following conclusions:

1. Chinook salmon fingerlings excrete principally ammonia and urea.

2. The patterns of excretion and composition of the excretory products over a 24-hour cycle vary with the density of the fish. At stocking rates of less than 5 pounds per gallon per minute of inflow, urea is the principal excretory product. Above this level ammonia becomes dominant.

3. Concentrations of un-ionized ammonia as low as 0.006 p.p.m. in continuous exposure for 6 weeks can produce extensive hyperplasia of the gill epithelium.

4. No deleterious effects were discernible when chinook fingerlings were subjected to continuous exposure to urea for 3 weeks at levels normally encountered in rearing ponds.

5. Controlled experiments have demonstrated that prolonged but intermittent exposure to un-ionized ammonia results in reduced growth rate and physical stamina. It is postulated that continuous ammonia exposure is the precursor of bacterial gill disease.

6. The pH and water temperature determine the amount of un-ionized ammonia present; the pond type and the

loading rate in relation to water inflow determine the duration of the exposure period.

7. Where ammonia is a problem, dangerous levels are indicated by the presence of measurable amounts in the effluent during the low ebb of the diurnal excretory cycle.

## LITERATURE CITED


BROCKWAY, DONALD R.
1950. Metabolic products and their effects. U.S. Fish and Wildlife Service, Progressive Fish-Culturist, vol. 12, No. 3 (July), p. 127–129.

BURROWS, ROGER E., and HARRY H. CHENOWETH.
1955. Evaluation of three types of rearing ponds. U.S. Fish and Wildlife Service, Research Report 39. 29 p.

DOWNING, KATHLEEN M., and J. C. MERKENS.
1955. The influence of dissolved-oxygen concentrations on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). Annals of Applied Biology, vol. 43, No. 2, p. 243–246.

ELLIS, M. M., B. A. WESTFALL, and MARION D. ELLIS.
1946. Determination of water quality. U.S. Fish and Wildlife Service, Research Report 9, 122 p.

FOLIN, OTTO.
1933. Standardized methods for the determination of uric acid in unlaked blood and in urine. Journal of Biological Chemistry, vol. 101, p. 111–125.
1934. The preparation of sodium tungstate free from molybdate, together with a simplified process for the preparation of a correct uric acid reagent (and some comments). Journal of Biological Chemistry, vol. 106, No. 1, p. 311–314.

FOLIN, OTTO, and HSIEN WU.
1919. A system of blood analysis. Journal of Biological Chemistry, vol. 38, No. 1, p. 81–110.

KARR, WALTER G.
1924. A method for the determination of blood urea nitrogen. Journal of Laboratory Clinical Medicine, vol. 9, p. 329.

KAWAMOTO, N. Y.
1961. The influence of excretory substances of fishes on their own growth. U.S. Bureau of Sport Fisheries and Wildlife. Progressive Fish-Culturist, vol. 23, No. 2 (April), p. 70–75.

KAWAMOTO, NOBU YUKI.
1958. Fresh water fish culture from the physiological point of view—I. Influences of ammonia nitrogen excreted by fishes on their growth in the culture ponds. Report of Faculty of Fisheries, Prefectural University of Mie, vol. 3, No. 1, p. 104–121.

KAWAMOTO, NOBUYUKI Y., YOSHIO INOUYE, and SUTEKICHI NAKANISHI.
1957. Studies on effect by the pond-areas and the densities of fish in the water upon the growth rate of carp (*Cyprinus carpio* L.). Report of Faculty of Fisheries, Prefectural University of Mie, vol. 2, No. 3, p. 437–447.

LLOYD, R., and D. W. M. HERBERT.
1960. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). Annals of Applied Biology, vol. 48, No. 2, p. 399–404.

MERKENS, J. C., and K. M. DOWNING.
1957. The effect of tension of dissolved oxygen on the toxicity of un-ionized ammonia to several species of fish. Annals of Applied Biology, vol. 45, No. 3, p. 521–527.

PHILLIPS, ARTHUR M., JR., DONALD R. BROCKWAY, MAURICE BRYANT, E. O. RODGERS, and JOHN MAXWELL.
1949. Metabolic product accumulation and its effect. New York State Conservation Department, Fisheries Research Bulletin No. 13, Cortland Hatchery Report No. 18, p. 19–20.

PHILLIPS, ARTHUR M., JR., DONALD R. BROCKWAY, ALBERT J. KOLB, and JOHN M. MAXWELL.
1950. The effect of metabolic products on brook trout. New York State Conservation Department, Fisheries Research Bulletin No. 14, Cortland Hatchery Report No. 19, p. 14–16.

SMITH, HOMER W.
1929. The excretion of ammonia and urea by the gills of fish. Journal of Biological Chemistry, vol. 81, p. 727–742.

THOMAS, ALLAN E., ROGER E. BURROWS, and HARRY H. CHENOWETH.
1964. A tunnel for stamina measurement of fingerling salmonids. U.S. Bureau of Sport Fisheries and Wildlife, Research Report 67. (In press.)

Webster's New International Dictionary of the English Language.
1951. William Allan Neilson [editor]. G. & C. Merriam Company. Second Edition, unabridged, 3214 p.

Wood, J. D.
1958. Nitrogen excretion in some marine teleosts. Canadian Journal of Biochemistry and Physiology, vol. 36, No. 12, p. 1237–1242, December.

Wuhrmann, Von K., and H. Woker.
1949. Beitrage zur toxikologie der Fische II, Experimentelle Untersuchungen uber die ammoniak-und blausaurevergiftung. Schweizerische Zeitschrift fur Hydrologie, vol. 11, p. 210–244.

U.S. GOVERNMENT PRINTING OFFICE : 1964 O—743-648

Created by act of Congress in 1849, the Department of the Interior is responsible for a wide variety of programs concerned with the management, conservation, and wise development of America's natural resources. For this reason it is often described as a department of natural resources.

Through a score of bureaus and offices the Department is responsible for the use and management of millions of acres of federally owned lands; administers mining and mineral leasing on a sizable area of additional lands; irrigates reclaimed lands in the West; manages giant hydroelectric power systems; administers grazing and forestry programs on federally owned range and commercial forest lands; protects fish and wildlife resources; provides for conservation and development of outdoor recreation opportunities on a nationwide scale; conserves hundreds of vital scenic, historic, and park areas; conducts geologic research and surveys; encourages mineral exploration and conducts mineral research; promotes mine safety; conducts saline water research; administers oil import programs; operates helium plants and the Alaska Railroad; is responsible for the welfare of many thousands of people in the territories of the United States; and exercises trusteeship for the well-being of additional hundreds of thousands of Indians, Aleuts, and Eskimos, as well as being charged with resource management of millions of acres of Indian-owned lands.

In its assigned function as the Nation's principal natural resource agency, the Department of the Interior bears a special obligation to assure that our expendable resources are conserved, that renewable resources are managed to produce optimum yields, and that all resources contribute their full measure to the progress, prosperity, and security of America, now and in the future.

UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
WASHINGTON, D.C. 20240

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF THE INTERIOR